## MICROBIAL DESULFURIZATION OF SOME EUROPEAN COALS: PROGRESS REPORT ON A JOINT ITALIAN PROJECT

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#### 1. INTRODUCTION

The continuing fluctuation of the oil market together with the likelihood of a rise in the current relatively low prices, highlight the ever-present problem of alternative energy resources availability. This problem, of worldwide impact, is particularly pressing in those industrialized countries which, like Italy, can rely on only very limited domestic energy resources and, on account of political decisions, must refrain from resorting to nuclear power.

In the strive towards the exploitation of all available domestic energy resources, The Italian state-owned energy supply company, ENI-AGIP, has reopened the Seruci mine in the Sulcis subbituminous coal basin (Island of Sardinia). The mine is expected to reach full production of two million tonnes per year of marketable coal by 1992. The entire production is intended for the electrical power stations operating in Sardinia. Sulcis coal has the characteristics shown in Table 1: its major drawback is its high sulfur content. Current legislation on industrial air pollution in Italy restricts peak concentrations of  $SO_2$  in power station emissions to 657 micrograms per cubic meter over 24 hours. Even more stringent are the limits suggested by the European Economic Community (EEC). These standards all impose severe limitations on the sulfur contents of coals since they virtually equate to maximum permissible sulfur contents of about 0.8%.

On the other hand, the sulfur contents of world coal reserves vary from 0.38 to 10%, and that of mineable reserves from 0.38 to 5.32%, which means that a considerable portion of the coal mined throughout the world cannot be directly used for combustion without some preliminary measures being taken to keep, in one way or another, emissions within the prescribed limits. Table 2 shows the pollution potential, in terms of grams of  $\mathrm{SO}_2$  per MJ of typical coals extracted from European and American mines.

Although most coal mined in the EEC is presently extracted from those parts of the respective basins containing low-sulfur coals, (i.e. coals containing less than 1% total sulfur), the economic importance of the possibility of mining high-sulfur coals too has been given full recognition by the EEC. Thus, in 1986, the EEC launched an R & D programme with the objective of assessing the possibilities offered by biohydrometallurgy in microbial coal desulfurization. This programme is now well under way and four countries are participating therein: Holland (Biotechnology Delft), Italy (University of Cagliari), the United Kingdom (Warren Springs Laboratories, Stevenage) and West Germany (Bergbau-Forschung GmbH).

In Italy the programme is also partly supported by the Italian company ENICHEM-ANIC, which, in 1986, signed a research contract with the Mining and Mineral Dressing Department (MMDD) of Cagliari University. Part of this research project has been entrusted to researchers of the Microbiology Institute of the 'La Sapienza' University (MILSU) in Rome, which is thus closely cooperating with the MMDD of Cagliari University.

The present paper is intended as a progress report on the problems encountered and the results achieved by the two above-mentioned Italian universities.

### 2. SULFUR COMPOUNDS AND THEIR IDENTIFICATION

Coal contains sulfur in four forms: metallic sulfides, with a predominance of iron sulfides ('pyritic sulfur'), atoms covalently bound in the organic sulfur compounds contained in the coal matrix ('organic sulfur'), iron, calcium sulfates and elemental sulfur. Pyritic and organic sulfur are the most common: elemental sulfur does not usually assay more than 0.2% and sulfates more than 0.1%. Moreover, being soluble in water, the sulfates can be readily removed during coal washing. Whilst pyritic sulfur can be very accurately identified with several procedures (reflected light microscopy, ordinary chemical analysis), much less is known about the nature of the organosulfur compounds forming the coal matrix and it is still the object of intensive research and debate. As a matter of fact, there are no reliable, direct and single methods for quantitatively determining the organosulfur compounds in coal and even their qualitative determination is considered by some workers to suffer from too many uncertainties (1). It has been suggested that organic sulfur is present in coal in at least five main forms, which correspond to the functional groups containing aliphatic or aromatic thiols, aliphatic, aromatic or mixed sulfides and/or disulfide heterocyclic compounds containing the thiophenic ring and the gamma-thiopyrone system (2),(3).

Although no evidence exists of the direct determination of these compounds, the well known diagram of Figure 1 has been proposed (4): it represents the molecular structure of a typical bituminous coal where the sulfur atoms bound in the organic molecules are indicated with arrows. According to Meyer (2), the average pyritic sulfur content of the world's coal reserves ranges from 0.09 to 3.97% and the organic sulfur content from 0.29 to 2.04%. In some high-sulfur coals, like those investigated herein, these percentages can be higher with predominance of organic sulfur. Hence, coal depyritization, be it carried out microbially or by other means, can only produce coals complying with environmental specifications when organic sulfur is negligible. Otherwise, the organic sulfur must necessarily be removed.

For the time being organic sulfur removal can only be attempted by chemical processing (5): however, the costs involved are prohibitive and discourage commercial application. These considerations warrant the investigation of the possibilities of microbial organosulfur removal.

#### 3. MATERIALS AND METHODS

#### 3.1 The Investigated Coals

The programme entails the investigation of the amenability to microbial desulfurization of four coals: Sulcis coal, from the Sulcis coal basin in southwestern Sardinia, Monopol coal, from the Monopol coal mine in West Germany, Gardanne coal, from Provence in southern France and a Spanish coal from the Teruel mine. At the time of writing the Teruel coal had not yet been delivered to Cagliari's laboratory. Tables 1, 3 and 4 summarize the most salient characteristics of the above-mentioned coals.

## 3.1.1. Sulcis coal

On the grounds of 'rank' determinations based on the average reflecting power of vitrinite, Sulcis coal can be considered a subbituminous coal according to the ASTM (USA) classification or a 'Glanzbraunkohle' according to the DIN (West Germany) classification. If the additional parameter represented by the upper thermal power - determined on pure coal and with its intrinsic moisture, in compliance with the latest EEC guidelines - is considered, then Sulcis coal can be placed among the 'average rank' coals, near to the limit of 'lower rank' coals. Its maceral composition is characterized by an abundance of the 'vitrinite' group, which in the I Seam ranges from 70 to 75%. After the vitrinite group, the maceral group exinite-liptinite is the most represented, with cutinite predominating: in the I Seam, the exinite-liptinite associa-

tion amounts to 12 to 24%. The maceral group inertinite ranges from 2 to 7%. The main associated mineral phases are dolomite, calcite, pyrite, marcassite, quartz, aragonite and clay.

Pyrite, which is present throughout the Sulcis coal, is syngenetic and finely dispersed within the coal matrix (Fig. 2), where it occurs as individual crystals from submicron to micron size, along with less frequent framboids no larger than 40-50 micrometers. This very fine intergrowth makes pyrite removal with classical physical mineral dressing methods impossible. Pyritic sulfur is always accompanied by higher proportions of organic sulfur.

## 3.1.2 Gardanne coal

The 'rank' and technological determinations indicate that this coal belongs to the class of 'subbituminous' coals (ASTM). The Gardanne coal is predominantly composed of vitrinite (up to 65%) and, to a lesser extent, of inertinite (inertodetrinite, semifusinite, fusinite) and exinite-liptinite (cutinite, resinite, sporinite). It is characterized by abundant occurrence of pyrite and carbonate phases (dolomite and calcite) accompanied by quartz and clay.

Pyrite, basically syngenetic in nature, is finely dispersed within the coal matrix in the form of individual crystals (sometimes submicronic in size) and of framboidal clusters ranging in size from 5 to 40 micrometers (Figure 3).

Organic sulfur is always present in high percentages: investigations with the electron microprobe revealed that vitrinite contains more organic sulfur than other macerals.

## 3.1.3 Monopol coal

This is a bituminous coal containing, in decreasing order, vitrinite, exinite and inertinite. Vitrinite occurs as bands of variable thickness, alternated with exinitic and inertinitic bands. The exinite group is predominantly composed of sporinite, cutinite and resinite, whereas the major components of inertinite are fusinite and semifusinite.

Pyrite occurs in Monopol coal in both the syngenetic and epigenetic form. The syngenetic pyrite appears as individual framboids ranging in size from a few micrometers up to 40 micrometers, with a predominance of the 20 micrometer size, and less frequently as groups of framboids. Epigenetic pyrite is present in the form of fissure fillings (Fig. 4) and is frequently accompanied by marcassite. The veins of epigenetic pyrite range in thickness from 20 to 300 micrometers, with 100 micrometers prevailing.

Pyrite is also present in cellular cavities of semifusinite and fusinite structures. Of the carbonates, calcite, and to a lesser extent siderite, can be mentioned. Quartz and clay were also detected.

#### 3.2 The Microbial Strains

### 3.2.1 Pyritic sulfur removal

For pyrite removal a <u>Thiobacillus ferrooxidans</u> active mixed culture was used, isolated from acid drippings of the Fenice Capanne Mine (Tuscany, central Italy) (6) routinely maintained in Cagliari's laboratory in Silvermann and Lundgren 9K medium.

### 3.2.2 Organosulfur removal

To the best of the authors' knowledge, only one strain of <a href="Pseudo-monas">Pseudo-monas</a> sp., denoted CBl strain and listed as ATCC No. 39381, obtained by chemical mutagenesis of a wild strain, is claimed to be so effective in removing some organosulfur compounds from coal (7), as to encourage its testing with a view to commercial application. Sulfolobus acidocaldarius is also claimed to be capable of removing some organic sulfur from coal, although it does not perform as well (8). The CBl strain and <a href="Pseudomonas putida">Pseudomonas putida</a> PAW 340 were therefore also investigated, at least for comparative purposes.

A campaign of microbial strains collection was undertaken with the aim of isolating microorganisms present in a model mining environment. The following procedure was adopted for isolating microorganisms from the Seruci mine: a 20 dm³ Mariotte carboy, containing the suitable culture medium, was placed on a platform supported by a steel arch of a gallery located at 250 meters below sea level, where the temperature was 35°C. The medium was allowed to spread and percolate over the coal exposed on the gallery wall and was finally collected in another carboy placed on the gallery floor (Fig. 5). Repeated samplings, carried out with complete and minimal media as well as minimal media supplemented with selective agents, led to the isolation of the following strains:

- from complete medium <u>Bacillus</u> sp., <u>Enterobacter</u>, sp. and <u>Micrococcus</u> sp.;
- from minimal medium, and minimal medium supplemented with dibenzothiophene (DBT) as sole carbon source, several Gram-positive and Gram-negative bacteria, with a prevalence of strains belonging to <a href="Bacillus">Bacillus</a> and <a href="Pseudomonas">Pseudomonas</a> genera.

The isolated microorganisms were serially subcultured at  $30^{\circ}$ C and  $26.17 \text{ rad.s}^{-1}$  in  $300 \text{ cm}^{3}$  flasks containing  $50 \text{ cm}^{3}$  of basal salt

solution M9 (9), supplemented with 5% powdered coal or a minimal medium supplemented with DBT).

# 3.2.3 Leaching techniques

All the sulfur removal tests were carried out in 300 cm<sup>3</sup> Erlenmeyer flasks containing either organosulfur compounds presumably present in the investigated coals (e.g. DBT) or coal along with the suitable culture medium. They were incubated on giratory shakers operated at the speeds and temperatures specified in the descriptions of the individual runs.

### 3.2.4 Reagents

All reagents used in the tests were analytical grade. The culture media were always made up with distilled water and, for laboratory testing, were always sterilized.

## 3.2.5 Analytical methods

Total sulfur was determined by means of the SC132 Instrument manufactured by LECO (St. Joseph, MI, USA). Pyritic sulfur was determined by gravimetric analysis, whereas iron and other elements were analysed by complexometric titration. Organic sulfur was determined as the difference between total and pyritic plus sulfate sulfur. The analyses of the residual DBT after microbial attack were carried out with an original method developed by the Industrial Chemistry Department of Bologna University (10).

Hydrogen ion concentrations were determined by means of potentiometric pH-meters of various makes (Beckmann, Orion and Hanna) and redox potential measured with a Mod. HI 8418 Hanna electronic potentiometer with Pt combined electrode.

## 3.2.6 Microbial growth measurements

Cell concentrations were obtained by the dilution plating method and by counting colony-forming units (CFU) per cm  $^3$ .

#### 4. RESULTS

### 4.1 Pyritic sulfur removal

Tests were carried out on Sulcis, Monopol and Gardanne coals, the coal from Teruel (Spain) being unavailable.

#### 4.1.1 Sulcis coal

All tests were performed in 300 cm3 Erlenmeyer flasks with baffled bottom, containing suspensions of coal powder in 9K medium. The shaker speed was adjusted to 26.3 rad.s-1 and temperature maintained at 28±1°C. CO2 was added to the shaker atmosphere. Owing to the very fine intergrowth of pyrite within the coal matrix and in spite of the relatively high porosity of the coal, a satisfactory access of the solutions and/or microbes to the pyrite crystals can only be achieved provided that most of them are at least partially exposed. Hence grinding to -40 micrometers is required to achieve 90% pyritic sulfur removal in a leaching time ranging from 9 to 12 days. These pyrite removals were achieved for solids-to-liquid ratios (weight of coal divided by weight of liquid medium) ranging from 1:100 to 16:100. Poorer results were obtained for solids-toliquid ratios higher than 20:100. The initial pH of the medium was adjusted to between 2.20 and 2.25, and pulp acidity had to be stabilized prior to inoculation with about 45 Kg of concentrated sulfuric acid per Mg of coal. The final pH of the leach liquor was usually 1.8. The redox potential of the medium steadily increased from about 370 mV to more than 650 mV.

### 4.1.2 Monopol coal

Several runs were carried out in the same conditions as those adopted for Sulcis coal, but the results were quite deceptive. Some runs lasted up to 25 days, but no appreciable pyrite removal was observed. The pH of the leach liquor only dropped very slightly (from 2.20 to 2.00) and the redox potential of the solution never rose above 540 mV. Viability tests showed however that microorganisms remained viable well after conclusion of the tests.

Factorial variance analyses are presently under way, with potassium, phosphorus and ammonium sulfate concentrations of the medium as variables.

### 4.1.3 Gardanne coal

A first series of tests was carried out in the same experimental conditions as those adopted for Sulcis coal. Pyrite removal appears to be of the same order of magnitude, but always lower, as that achieved with Sulcis coal: the solubilization rate is constantly about 20% slower; Preliminary tests carried out on this coal varying phosphorus, potassium and ammonium sulfate concentrations in the medium, seem to indicate that a suitable composition of the medium might considerably improve the effectiveness of microbial attack.

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# 4.2 Organic Sulfur Removal

All the isolated strains were analysed in order to assess their ability to grow in basal salt solution supplemented with powdered coal (5% w/v) or a selective agent (1 per thousand DBT w/v). The data obtained show that all strains can grow on basal salt solution supplemented with coal. On the other hand, only the strains preselected in the mine using basal salt solution plus DBT exhibited significant growth when cultivated in the presence of this selective agent.

As for coal-degrading activity, removal of between 10 and 18% organic sulfur was observed. Under these experimental conditions, no degrading activity was detected for the CBl strain.

It should be noted that for sulfur contents of the order of magnitude of those of the examined coals (from 1.5 to 6.0%), a certain unpredictable variability was observed in the data provided by the described analytical techniques. This fact stresses the need for analytical methods of adequate sensitivity to detect fraction percent variations in sulfur contents, presently lacking.

The nutritional characteristics of the above-mentioned microorganisms are shown in Table 5.

## 5. REMARKS AND CONCLUSIONS

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The occurrence in several EEC countries of sizeable reserves or high-sulfur coals containing large proportions of organic sulfur as well as the economic potential of the high-sulfur coal zones exhibiting similar characteristics in several coal basins, provide an impetus for needed research aimed at removing both types of sulfur.

Microbial removal of pyritic sulfur is, in principle, a well-defined process, and the effectiveness of <u>Thiobacillus ferrooxidans</u> in dissolving the accessible pyrite from the coal matrix is well documented (11),(12),(13). However, the present research has emphasized the major role played by the chemical composition of the run-of-mine coal. The presence of phosphorus, potassium and nitrogen compounds may, in effect, alter the composition of the nutrient medium to such an extent as to diminish the effectiveness of microbial action, probably due to substrate inhibition. It is therefore advisable to always design the composition of the basal salts medium according to the type of processed coal.

Exposure - not necessarily liberation - of the pyrite component is an essential condition for its microbial removal. Hence, petro-

graphic and porosity analyses of the coal should always provide the information required to avoid excess of expensive grinding. As shown by the diagrammatic sketch of Fig. 6, porosity effectively contributes to the accessibility of solutions and/or microorganisms to pyrite crystals not exposed on the coal grain surface.

Information on microbial organosulfur removal is very scanty. Among the strains claimed in the literature (7),(8) to be effective to a certain extent are <u>Sulfolobus acidocaldarius</u> and <u>Pseudomonas</u> strain CBl obtained by chemically induced mutation, although little has been published on the organosulfur removal ability from coal. The tests carried out to date in the present investigation do not seem to support the effectiveness claimed for strain CBl in removing organic sulfur. On the other hand, the organic-sulfur degradation ability exhibited by the strains isolated from the mine environment, appears encouraging.

One hundred percent sulfur removal cannot be achieved inasmuch as the sulfur atoms are dispersed throughout the coal matrix and therefore only those exposed on the coal surface (grain boundaries or pore surfaces) can be removed. The larger the surface-to-volume ratio, the more the sulfur is amenable to microbial attack. However, the surface-to-volume ratio cannot be increased beyond a certain value, owing to the high grinding costs and to the depreciation of coal when its grain size is smaller than market specifications. For the time being, the finest coal particles find commercial application in the coal-water mixtures technique, with an average grain size of 40 micrometers.

It is therefore imperative to exploit other means for increasing the exposed surface. One possibility is preliminary depyritization and dissolution of the carbonate phases present in coal (Fig. 7): the surfaces of all the cavities left by pyrite and carbonates removal contribute to coal matrix, and hence organic sulfur, exposure. On these grounds, a tentative flowsheet for microbial coal desulfurization is proposed in Fig. 8. This flowsheet should be considered, at the present state of progress of the research, as a guideline for the planning of laboratory and pilot plant testing.

It should finally be pointed out that the present inability of analytical chemistry to provide reliable analytical methods for quantitatively determining the organosulfur compounds forming the coal matrix contributed to the difficulties encountered in evaluating the organic sulfur removal by the above-mentioned microorganisms.

### ACKNOWLEDGEMENTS

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#### REFERENCES

- 1) BOUDOU, J.P., <u>In verbis</u>, 1988.
- 2) MEYERS, R.A., <u>Coal Desulfurization</u>, Marcel Dekker, Inc., New York, 1977.
- 3) CHENG TSAI, S., Sulfur in coal and reactions of sulfur compounds related to coal desulfurization, in <u>Fundamentals of Coal Beneficiation and Utilization</u>, Elsevier, Amsterdam, 1982, 222 (Chap. 5).
- 4) WHEELOCK, T.D., An overview of coal cleaning technology, in Proceedings of the Coal Water Fuel Technology Workshop, U.S. Dept. Energy Tech. Ctr., Rept. No. BNL 51427, 1981, 82-101.
- 5) MORRISON, G.F., Chemical desulphurization of coal, Report No. ICTIS/TR15, June 1981, IEA Coal Research, London.
- 6) ROSSI, G., La lisciviazione microbica dei minerali. I. L'azione dei microorganisms reperiti in acque acide di miniere italiane sulla pirite, Res. Ass. Min. Sarda, Iglesias (Italy), 76, 5-23, 1971.
- 7) ISBISTER, J.D. and KOBYLINSKY, E.A., Microbial desulfurization of coal, in <u>Processing and Utilization of High Sulfur Coals</u>, ATTIA, Y.A., Ed., Elsevier, Amsterdam, 1985, 627-641.
- 8) KARGI, F. and ROBINSON, J.M., Removal of organic sulphur from bituminous coal. Use of the thermophilic organism <u>Sulfolobus</u> acidocaldarius, Fuel, 65, 397-399, 1986.
- MANIATIS, T., FRITSCH, E.S. and SAMBROOK, J., Molecular cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1983.
- 10) PIFFERI, P.G., In verbis, 1988.
- 11) DETZ, C.M. and BARVINCHAK, G., Microbial desulfurization of coal, Min. Cong. J., 65, 75-82 and 86, 1979.
- 12) HOFFMANN, M.R., FAUST, B.C., PANDA, F.A., KOO, H.H. and TSUCHIYA, H.M., Kinetics of the removal of iron pyrite from coal by microbial catalysis, Appl. Environ.Microbiol., 42, 259-271, 1981.

13) HUBER, T.F., KOSSEN, N.W.F., BOS, P. and KUENEN, J.G., Modelling, design and scale-up of a reactor for microbial desulphurization of coal, in <u>Progress in Biohydrometallurgy</u>, ROSSI, G. and TORMA, A.E., Eds., Ass. Min. Sarda, Iglesias (Italy), 1983, 279-289.

Table 1 - Data of a typical Sulcis coal

Analysis of macerals	Chemico-technol analysis	ogical	Reflective power of vitrinite		
Vitrinite 76.4	Ash	7.7 %	0.49		
Exinite 15.8	Sulfur, total	5.9 %			
Inertinite 3.8	Pyritic sulfur	1.06%			
	Sulfate sulfur	0.8 %			
	Iron, total	1.4			
	CaO	20.5 % of ash			
	K <sub>2</sub> O	n.đ.			
	MgO	11.2 % of ash			
	P <sub>2</sub> O <sub>5</sub>	0.8 % of ash			
	Heating power (dry basis)	20.485 kJ.kg <sup>-1</sup>			
	Porosity	31.46			

n.d. = not detected

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<u>Table 2 - Examples of average sulfur, ash and energy content of several coal samples</u>

Country/State	County	Coal Seam	kJ/kg	% Ash	Total S %	g SO <sub>2</sub> per MJ
France		Gardanne	28,360	10.84	5.67	4.0
Italy	Sulcis	I Seam	27,900	10.81	6.46	4.6
Spain		Berga	15,900	41.0	4.75	6.0
		Utrillas	21,850	18.6	7.92	7.2
USA						
-Ohio	Belmont	Pittsburgh	28,600	15.1	6.19	4.3
	Coshocton	Middle Kittanning	28,360	14.1	6.39	4.5
	Muskingum	Lower Kittanning	28,800	11.5	4.85	3.4
-Pennsylvania	Indiana	Lower Kittanning	31,380	12.9	4.68	3.0
-Utah	Emery	Blind Canyon	31,850	5.6	0.58	0.3
-Virginia	Wise	Taggart	33,940	2.9	0.79	0.5
-W.Virginia	Preston	Upper Freeport	28,800	18.5	2.44	1.7
•	Nicholas	Middle Kittanning	27,900	20.2	1.06	0.8
-Wyoming	Sweetwater	Rock Spring	29,060	6.5	0.97	0.7

Table 3 - Chemico-technological characteristics of Monopol coal

Component	Weight %	Porosity %		
Ash	28.5	4.5		
Sulfur, total	1.97			
Pyritic sulfur	1.33			
Sulfate sulfur	0.21			
Iron, total	2.6			
Carbonates	0.4	% of ash		
-K <sub>2</sub> O	4.4	% of ash		
-Na <sub>2</sub> O	1.1	% of ash		
-MgO	1.9	% of ash		

Table 4 - Chemico-technological characteristics of Gardanne coal

Component	Weight % 			Porosity % 29.09		
Ash						
Sulfur, total	5.1					
Pyritic sulfur	1.06					
Sulfate sulfur	0.21					
Iron, total	0.7					
Cao	20.0	8	of	ash		
K <sub>2</sub> O	0.3	ક્ર	of	ash		
MgO	3.4	8	of	ash		
P <sub>2</sub> O <sub>5</sub>	2.3	ક	of	ash		

Table 5 - Nutritional characteristics of microorganisms isolated in Seruci coal mine

				M9 supplemented with			
Colony	Isolation !	Medium	Glucose 2%	NaBenzoate 1.5%	NaBenzoate 1.5%+DBT 1%	DBT 1%	
ln	M9+glucose	2%	+	+	+	+	
2P	M9+NaBenz.	1.5%	+	+	+	+	
1Q	M9+NaBenz.	+DBT	+	+	+	+	
lR	M9+DBT	1%	+	+	+	+	
1H	McConkey		+	+	+	+	
1A	LB		-	-	+		
18	LB		+	+	+	_	
1s	MMA+coal	5%	+	+	+	-	
lL	M9+glucose	2%	+	-	+	-	
lM	M9+glucose	2%	+/-	-	-	+	
2A	LB		+		-		

l = strains isolated in mine by means of M9+DBT

<sup>2 =</sup> strains isolated in mine by means of M9

N.B. The strains capable of growing only in the presence of glucose as carbon source are not reported, except 2A, which has been used as control.

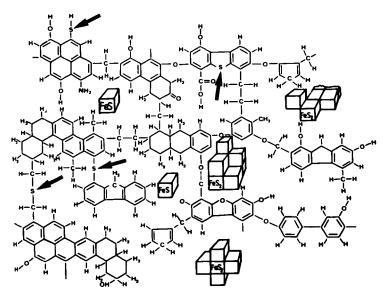


Figure 1 - Molecular structure of a typical bituminous coal after Wendell Wiser. The sulfur atoms bound in organic molecules are indicated by arrows.(Modified from Wheelock ( )).

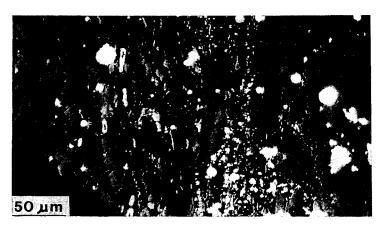
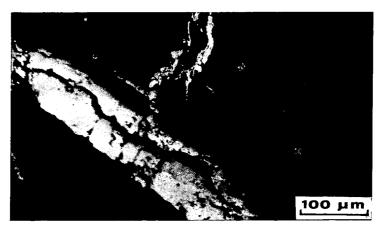


Figure 2 - Pyrite in Sulcis coal: fine dispersions; refl.light, oil immersion.

50 µm

Figure 3 - Pyrite in Gardanne coal: framboids; refl. light, oil immersion.



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Figure 4 - Pyrite in Monopol coal: epigenetic veins in cleats; refl.light, oil immersion.

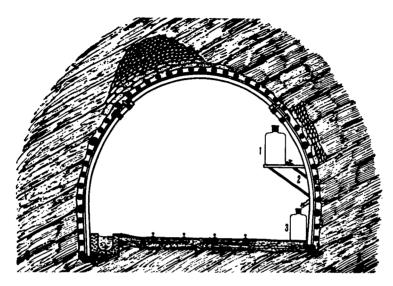


Figure 5 - Set-up for collecting microbes in the Seruci mine.  $1 = \text{head tank}; \quad 2 = \text{pipe}; \quad 3 = \text{medium collecting carboy.}$ 

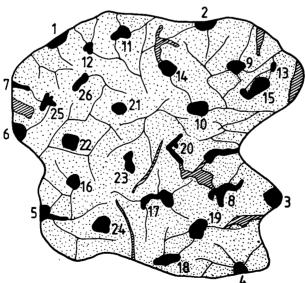


Figure 6 - Two-dimensional model of coal particle. Black grains are pyrite; dashed grains are carbonates; white matrix is coal; lines are pores or cracks.

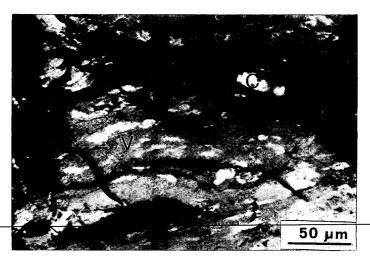


Figure 7 - Sulcis coal: carbonates (C) in fissures in vitrinite (V); refl.light, oil immersion.

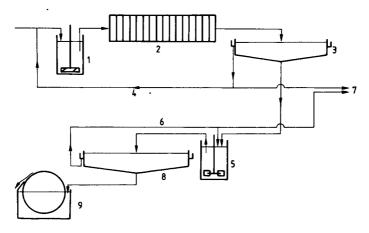


Figure 8 - Microbial coal desulfurization flowsheet. 1: backmix reactor; 2: plug flow reactor for pyrite removal; 3: thickener; 4: medium recycle; 5: backmix reactor for organic sulfur removal; 6: supernatant to waste; 7: tailings disposal; 8: thickener; 9: filter.